
Functional Genomics and Abiotic-Stress Tolerance in Cereals

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Environmental or abiotic stresses such as high temperature, low water availability, salinity and mineral toxicity and deficiency frequently affect plants in agricultural systems, and represent major limitations to the yield and quality of barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L. and *turgidum* L.) crops. It is common for many abiotic stresses to challenge a crop simultaneously. For example, the occurrence of high temperatures is common during periods of limited water availability, and often under these conditions plant roots encounter high concentrations of salt and boron in the subsoil.

Grain yields in Australia are vulnerable to climatic variation. This is evident from wheat yields over the past decade, which have ranged from approximately 1.1 to 2.1 t/ha. In 1982, 1994 and 2002, major droughts (high temperatures, low rainfall) across southern Australia had drastic effects on cereal production, with less than 10 million tonnes of wheat produced in these years. This is in contrast to favorable years, such as the 1983/84 season following the 1982 drought, which produced a wheat crop of 22 million tonnes. In recent years, the area planted to cereal crops in Australia has increased, largely as growers switched from sheep farming due to decreasing wool prices. Average wheat yield per hectare has also been rising, at a rate of approximately 1.6% per year over the past 20 years.

Historically, improvement of tolerance to abiotic stresses has been a major target of plant-breeding programs globally. The major challenge, however, results from the complex nature of abiotic-stress-tolerance traits and the difficulty in dissecting them into manageable genetic components amenable to molecular breeding. In crop breeding, advances in molecular biology and genomics have had a large impact on the speed of identification and characterization of genes and genetic regions associated with quantitative and qualitative traits. Marker-assisted selection through the use of high-throughput marker systems is currently being used extensively in breeding programs to improve selection efficiency, accuracy and to direct focus towards traits of importance. As key genes are identified, efficiency increases and opportunities for genetic engineering are realized. An underlying factor important for gene discovery in relation to traits of interest is naturally occurring genetic diversity. This is a fundamental aspect of research into abiotic-stress tolerance, and discoveries of abiotic-stress-tolerance genes in cereals is revealing novel mechanisms of

adaptation in crop plants and their wild relatives. Genetic-diversity screening is a starting point for many functional genomics projects relating to gene discovery. The extensive genetic diversity available within the grasses makes this an excellent system in which to work on abiotic-stress tolerance.

CASE STUDIES: ABIOTIC STRESSES OF IMPORTANCE TO THE SOUTHERN AUSTRALIAN CROPPING SYSTEM

Boron Toxicity

Boron is one of the eight elements that are essential for healthy plant growth. Its deficiency in crops is well known, and has been intensively studied. However, in semi-arid areas of the world such as Southern Australia, West Asia and North Africa, boron content in soils is high and can limit productivity. In South Australia, more than 30% of soils in grain-growing areas have levels of boron considered toxic to plant growth. Yield penalties of up to 17% between adjacent areas of barley have been attributed to differences in shoot-boron concentration, and similar figures have been reported for wheat (Cartwright *et al.*, 1984; Moody *et al.*, 1993). The primary mechanism of tolerance appears to be similar for all species studied: an ability to maintain low concentrations of boron in plant tissues (Fig. 1). Identification of the genes controlling this important trait have been a major focus of our work at the Australian Centre for Plant Functional Genomics (ACPFG).

Previous work at the University of Adelaide over more than two decades has resulted in the identification of the major genes involved in boron-toxicity tolerance in barley (Jefferies *et al.*, 1999; Sutton *et al.*, 2007) and wheat (Paull *et al.*, 1995; Jefferies *et al.*, 2000). The progression of this work follows a pathway that in many ways parallels technological development in areas of modern plant science, from traditional genetic studies determining the underlying genetic basis for tolerance, mapping studies to determine more accurately the chromosomal position of loci involved (also known as QTL, quantitative trait loci), physiological approaches to investigate the mechanisms at play, to the more-recent fields of functional genomics to identify candidate genes and clarify their roles. We recently identified the gene *Bot1* in barley, underlying the most significant QTL associated with boron-toxicity tolerance in this species. The locus has been the target of breeding programs for more than 15 years in Southern Australia, using marker-assisted selection. The jury is still out as to the success of this conventional introgression approach, as lines carrying the introgression can potentially be lower-yielding than the recipient cultivars. It is unknown if this is due to pleiotropic effects of the tolerance gene itself, or due to unwanted deleterious alleles from the otherwise unadapted, but boron-tolerant, donor landrace.

Identification of the gene involved now places us in an exciting position. Not only do we understand the molecular basis for tolerance, but we can now also provide breeding programs with lines carrying recombination events close to the tolerance gene, helping to break unwanted associations. We can also focus on the generation of boron-tolerant germplasm using genetic transformation. This work represented the first isolation of an abiotic stress QT locus in a cereal, and revealed novel concepts on gene duplication and evolution of tolerance to abiotic stress in cereals.

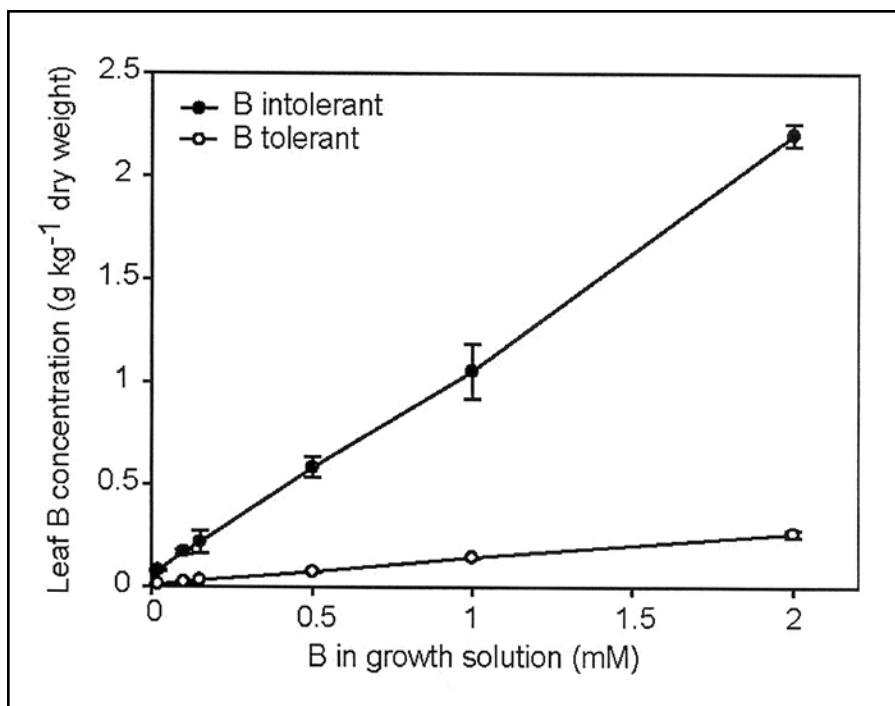


Figure 1. Genetic variation for boron tolerance in barley.

Salinity

Salinity is a major abiotic stress affecting crops in Australia and throughout the world. More than 800 million hectares of land are salt affected globally, accounting for more than 6% of the total land area (Munns and Tester, 2008). In Australia, some 20,000 farms may be affected by salinity: of 1,969,000 hectares of arable land affected by salinity, 820,000 may be unusable (ABS, 2002). Western Australia, the largest wheat- and barley-producing state in Australia, is the worst affected with yields on 51% of the state's farms constrained to some extent by saline soils. In most crop plants, the main toxic component of salinity is the sodium ion (Na⁺), which interferes with metabolic processes such as enzyme activity and protein synthesis, as well as causing osmotic stress through the reduced ability of cells to obtain and retain water. Due to these toxic effects, crops grown on saline soils have significantly reduced yields.

Plants use three main mechanisms with which to tolerate salinity stress (Munns and Tester, 2008):

- osmotic-stress tolerance, the ability to maintain growth under osmotic stress, a process that causes stomatal closure and reduced cell expansion in root tips and leaves,

- Na^+ exclusion, the reduction of Na^+ accumulation in shoots by Na^+ exclusion in the roots, and
- tolerance of tissues to accumulated Na^+ and possibly Cl^- , requiring, in most cases, compartmentation of Na^+ and Cl^- at the cellular and intracellular levels.

The focus of salinity research at ACPFG is diverse and covers aspects within these three areas. Functional genomics provides new opportunities to understand these processes in the plant, enabling the identification of genes involved and providing opportunities to generate plants that are able to survive and produce viable yields on saline soils.

Drought

Drought tolerance is a key trait of interest for cereal breeding. Predictions of human-population growth globally, and accompanying shortages of arable land and water supply emphasize a need for crop-plant development in this area. In Australia, drought stress continues to be a major factor affecting the productivity of our rain-fed cereal crops. Since 2001/02, much of Australia's most productive agricultural land, primarily in the southeast, has experienced conditions of higher-than-average temperatures and lower-than-average rainfall (ABARE, 2009). In 2007, after several preceding years of drought, we witnessed one of the hottest growing seasons on record across much of Southern Australia, with crop losses much larger than expected. This trend of declining rainfall and increasing temperatures is predicted to continue, emphasizing a need for scientific approaches to develop germplasm adapted to these hostile conditions.

Drought tolerance is a difficult trait to define as it encompasses a wide range of characteristics involving multiple genetic, physiological, cellular and biochemical strategies in the plant. Dissecting drought tolerance to the level of a single gene or group of genes amenable to genetic engineering will be difficult. A major challenge in the use of functional genomics to enhance the development of drought tolerance is to define the system and focus on key traits of interest. ACPFG has been tackling the drought problem using three strategies. The first is a forward genetic approach aimed at defining the genetic basis for differences in tolerance in adapted germplasm. The second aims to build a database of transcript, protein and metabolite responses of wheat and barley exposed to drought stress that can be used to support candidate-gene discovery. The third involves specific targeting of genes with known roles in drought-stress tolerance in other species, such as transcription factors and protein kinases.

One of our core activities involves the genetic analysis of crosses involving elite bread-wheat germplasm that display variation for grain yield under drought-stress conditions in Southern Australia. Figure 2 shows three advanced wheat lines, 'Kukri,' 'Excalibur' and 'RAC875,' from which we have established two large mapping populations. In both cases, 'Kukri' is the drought-sensitive parent, with 'RAC875' and 'Excalibur' representing two different mechanisms for drought response. The populations have now been characterized for more than forty phenotypic traits related to drought tolerance (Table 1) in twenty environments. The key objective of this work is the identification of QTL that influence a range of drought-related traits and ultimately yield under local conditions. The work

has identified the location of chromosomal segments associated with the maintenance of yield or yield components under drought-stress conditions. These regions are being targeted in other projects aiming at validation and ultimately the positional cloning of the genes involved.

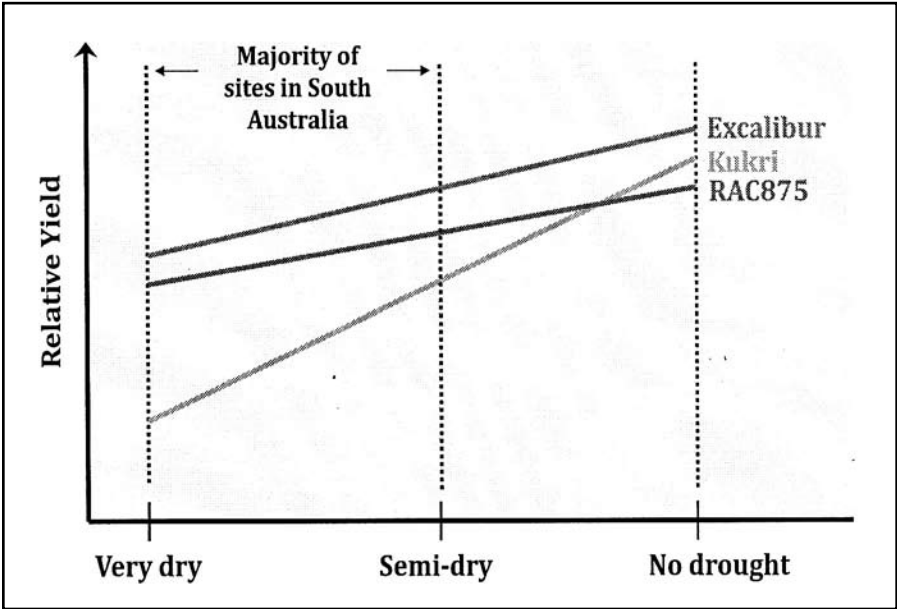


Figure 2. Genetic variation for drought tolerance in wheat (source: Steve Jefferies, Australian Grain Technologies).

PIPELINES FOR FUNCTIONAL ANALYSIS OF CANDIDATE GENES

A crucial part of the pipeline for gene discovery, characterization and downstream delivery of abiotic-stress-tolerant germplasm is the functional testing of candidate genes *in planta*. We have established a cereal-transformation facility that, in 2008, transformed wheat and barley with more than 100 constructs, generating in excess of 1,500 independent transgenic events. Of importance and key to our strategy is the ability to reliably and routinely transform elite breeding varieties of wheat and barley with genes relevant to key traits, such as drought tolerance. This provides a significant advantage in the testing of candidate genes, by allowing analysis of gene effects in otherwise highly adapted backgrounds. A significant contribution to the downstream analysis of material generated from our gene-discovery programs is the establishment of the Australian Plant Phenomics Facility (www.plantphenomics.org.au), directed by Professor Mark Tester (ACPF, University of Adelaide). The facility, due for completion in December 2009, will provide a state-of-the-art plant-growth complex, comprising 2,400 m² of glasshouse and 250 m² of growth-room space, allowing the analysis of more than 100,000 plants annually. The facil-

TABLE 1. A SELECTION OF DROUGHT-TOLERANCE TRAITS MEASURED IN WHEAT DROUGHT-MAPPING POPULATIONS (SOURCE: JAMES EDWARDS, ACPFG).

Development	Seed	Leaf	Root
Zadoks (maturity score)	Aborted florets	Leaf rolling	Crown rot
Degree days to heading	Fertile seeds/spike	Glaucousness (waxiness)	<i>Rhizoctonia</i>
Days to heading	Spikes/m ²	Canopy temperature	
Days to anthesis	Grains/m ²	NDVI (canopy reflectance)	
Days to senescence	1,000-grain weight	Water-soluble carbohydrates–content	
Early vigor	Screenings (<2 mm)	Water-soluble carbohydrates–per unit area (m ²)	
Plant height	Hectare litre weight	Water-soluble carbohydrates–per tiller at jointing	
Head length	Yield (kg/ha)	Water-soluble carbohydrates–per tiller at maturity	
Peduncle length		Tipping	
Number of plants at jointing		Flag-leaf length	
Number of tillers at jointing		Flag-leaf length	
Tillers/plants at jointing		SPAD chlorophyll-meter reading	
Number of tillers at maturity		Anthesis biomass	
Tillers/plant at maturity		Harvest index	
Aborted tillers		Maturity biomass	
Aborted tillers/plant			
Grain-filling duration			

ity will be based around automated image analysis; plants will be grown under controlled conditions and delivered to high-resolution imaging stations by a network of 1.2 km of conveyor belts. Accurate and detailed measurements of the phenotypic characteristics of germplasm collections, breeding populations, mutant populations and transgenic material will be possible. Non-destructive measurements will be possible of:

- shoot mass, leaf number, shape, angle, leaf color and senescence using visible-spectrum images,
- leaf water and carbohydrate content using near-infrared images,
- and leaf temperature using far-infrared images.

The Australian Plant Phenomics Facility will relieve a significant bottleneck in the area of plant phenotyping, limiting capability to capitalize on substantial investments in functional genomics and molecular breeding technologies in Australia.

CONCLUSION

Traditional molecular genetic studies have contributed greatly to our understanding of the underlying biology of tolerance to abiotic stresses. We know a great deal about the genetic control of traits for tolerance to major abiotic stress in cereal crops, such as boron tolerance, salinity tolerance, reproductive frost tolerance, and aspects of drought tolerance. This information has proven especially useful in breeding programs able to exploit molecular methods to aid selection. In some cases, the genes involved have been identified and their study is providing fascinating insight into adaptive-evolution mechanisms in plants. Gene identification also provides the opportunity to generate new varieties in the future via genetic transformation with superior abiotic-stress tolerance. Although genetically engineered wheat and barley are not yet grown on a commercial scale in Australia or other parts of the world, it may occur in the future. Functional genomics has a central role in this process, as part of a strategy to provide molecular solutions to pre-existing abiotic stresses, and also to address the prospective changes in our environment.

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SINCE 2003, Tim Sutton has been a research fellow and group leader at the Australian Centre for Plant Functional Genomics, a Commonwealth-funded entity focusing on the application of genomics to address drought, salinity and associated abiotic stresses that severely limit agricultural production in semi-arid environments.

The focus of Dr. Sutton's research is to understand the genetic and molecular mechanisms that enable some plants to survive under sub-optimal soil conditions, such as mineral toxicity and deficiency, and to develop cereals with increased tolerance to these stresses. The work involves collaboration within Australia and internationally, has attracted the attention of the popular press, and has been published internationally, including in the journal *Science* describing the first example of the cloning of an abiotic-stress-tolerance quantitative trait locus from a large-genome cereal. He has presented invited seminars in the United States, Europe and Egypt, and is regularly invited to peer review research papers for journals including the *Plant Journal*, *Genome*, and *Theoretical and Applied Genetics*.

In addition to supervision of graduate students in the area of cereal molecular biology, he is active in community and outreach activities within the Australian biotechnology education sector. He has a PhD in plant molecular genetics from the University of Adelaide (2002).